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Characterization of *Deinococcus radiodurans* for Actinide Precipitation

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We study *Deinococcus radiodurans*, a nonpathogenic prokaryote able to withstand high doses of ionizing radiation, for bioremediation and biostabilization of heavy metals and actinides to limit the migration of these contaminants. Our goal is to develop a system for *D. radiodurans* to bioprecipitate actinides similar to that of a genetically engineered strain of *Pseudomonas aeruginosa* capable of polyphosphate accumulation, inducible degradation and secretion, and UO_2^{2+} precipitation.

The radioresistance of *D. radiodurans* to ionizing gamma radiation has been well studied, yet is not well understood[1]. Several DNA repair mechanisms have been discovered, yet survivability studies are typically done under nutrient-rich conditions at a single growth stage. Although a minimal irradiation medium has been described[2], a clear study of the effect of different variables on survivability in a nutrient-starved environment has not been performed. We have shown that *D. radiodurans* grown in typical TGY complex media displays increasing radioresistance with increasing age of the culture at the time of harvest; however, the dose below which there is little loss of reproductive viability does not change significantly with growth phase. Higher irradiation rates in nutrient-starved medium display higher survivability. These results indicate that there is a threshold below which the radiation dose is not sufficient to overcome the cells' passive defense mechanisms, such as radical scavenging by carotenoids in the cell wall. Additional radiation resistance is then due to active resistance induced by cell damage, which can be related to aging. Using the 88" cyclotron at the Lawrence Berkeley National Laboratory, we also studied the effects of light-ion irradiation in aqueous suspension and show increasing lethality corresponding to increasing linear energy transfer (LET) values of the radiation. Previous studies only examined heavy ion effects on cells supported on solid medium[3].

To better understand the chemistry of non-engineered *D. radiodurans,* we studied the interaction of strain R1 with UO_2^{2+} in dilute salt solution. R1 sorbs uranyl more than two orders of magnitude less than the engineered *P. aeruginosa*. We are increasing the ability of *D. radiodurans* to sequester uranium via metabolic engineering. Chemical studies of the cell-uranyl binding strength and pH optima support spectroscopic data indicating that a carboxyl surface group, consistent with known characteristics of *D. radiodurans'* S-layer[4], interacts with and binds the uranyl. Studies including Infrared Spectroscopy, Laser Fluorescence Spectroscopy, and Extended X-ray Absorption Fine Structure Spectroscopy (EXAFS) are underway to further elucidate the mechanism of uranyl complexation to the cell surface and to further characterize a strain provided that has been engineered to accumulate polyphosphate.

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